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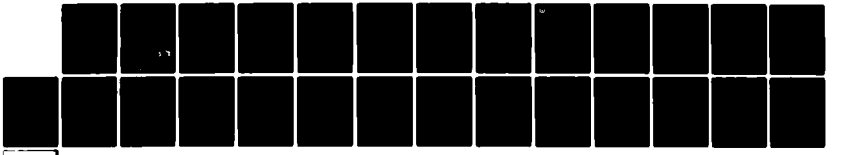

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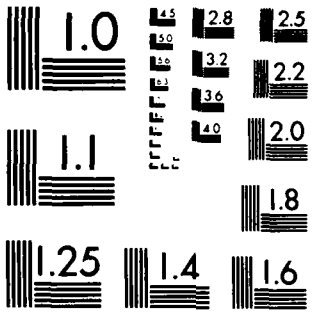

INSTITUTE REPORT NO. 146

AD A130158

MUTAGENIC POTENTIAL OF:

4-nitrophenyl monochloromethyl (phenyl) phosphinate (CMP)

LEONARD J. SAUERS, MS, SP5
and
JOHN T. FRUIN, DVM, PhD, COL VC

TOXICOLOGY GROUP,
DIVISION OF RESEARCH SUPPORT

MAY 1983

Toxicology Series 50

LETTERMAN ARMY INSTITUTE OF RESEARCH
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Toxicology Series 50--Sauers and Fruin

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The mutagenic potential of 4-nitrophenyl monochloromethyl (phenyl) phosphinate (CMP*) was assessed by using the Ames Salmonella/Mammalian Microsome Mutagenicity Assay. Tester strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 were exposed to doses ranging from 1 mg/plate to 3.2×10^{-4} mg/plate. It was determined that the test substance did not have mutagenic potential.

*Code number for compound

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ABSTRACT

The mutagenic potential of 4-nitrophenyl monochloromethyl (phenyl) phosphinate (CMP*) was assessed by using the Ames Salmonella/Mammalian Microsome Mutagenicity Assay. Tester strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 were exposed to doses ranging from 1 mg/plate to 3.2×10^{-4} mg/plate. It was determined that the test substance did not have mutagenic potential.

*Code number for compound

KEY WORDS: Mutagenicity, Toxicology, Ames Assay, 4-Nitrophenyl Monochloromethyl (Phenyl) Phosphinate (CMP).

PREFACE

TYPE REPORT: Ames Assay GLP Study Report

TESTING FACILITY: US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129

SPONSOR: US Army Medical Research and Development Command
US Army Medical Research Institute of Chemical Defense
Aberdeen Proving Ground, MD 21010

PROJECT: 35162772A875 Medical Defense Against Chemical Agents,
WU 304, Toxicity Testing of Phosphinate Compounds,
APC TL04

GLP STUDY NUMBER: 82024

STUDY DIRECTOR: COL John T. Fruin, DVM, PhD, VC, Diplomate of
American College of Veterinary Preventive Medicine

PRINCIPAL INVESTIGATOR: SP5 Leonard J. Sauers, MS

REPORT AND DATA MANAGEMENT: A copy of the final report, study protocols, raw data, retired SOPs and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: 4-nitrophenyl monochloromethyl (phenyl) phosphinate (CMP)

INCLUSIVE STUDY DATES: 10 August - 10 September 1982

OBJECTIVE: To determine the mutagenic potential of 4-nitrophenyl monochloromethyl (phenyl) phosphinate (CMP) using the Ames Assay. Tester strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 were used. The test substance was dissolved in dimethyl sulfoxide (DMSO) and this diluent was checked for sterility.

ACKNOWLEDGMENTS

The authors wish to thank SP4 Lawrence Mullen, BS; Carolyn M. Lewis, MS; and John Dacey; for their assistance in performing the research.

Signatures of Principal Scientists involved
in the Study

We, the undersigned, believe the study number 82024 described in this report to be scientifically sound and the results and interpretation to be valid. The study was conducted to comply, to the best of our ability, with the Good Laboratory Practice Regulations outlined by the Food and Drug Administration.

Leonard J. Sauers 23March83
LEONARD J. SAUERS, MS / DATE
SP5
Principal Investigator

23Mar83
JOHN T. FRUIN, DVM, PhD / DATE
COL, VC
Study Director

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MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

I hereby certify that in relation to LAIR GLP study 82024 the following inspections were made:

9 Aug 82
18 Aug 82
23 Aug 82

The report and raw data for this study were audited on 27 Apr 83.

Routine inspections with no adverse findings are reported quarterly, thus these inspections are also included in the Oct 82 report to management and the Study Director.

NELSON R. POWERS, Ph.D.
CPT, MSC
Quality Assurance Officer

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MUTAGENIC POTENTIAL OF: 4-nitrophenyl monochloromethyl (phenyl) phosphinate (CMP)
--Sauers and Fruin

Rationale for using the Ames Assay

The Ames Salmonella/Mammalian Microsome Mutagenicity Test is one of a standard bank of tests used by our laboratory for the assessment of the mutagenic potential of a test substance. It is a short-term screening assay, which we use for the prediction of potential mutagenic agents in mammals. It is inexpensive when compared to in vivo tests, yet is highly predictive and reliable in its ability to detect mutagenic activity and therefore carcinogenic probability (1). It relies on basic genetic principles and allows for the incorporation of a mammalian microsomal enzyme system to increase sensitivity through enzymatically altering the test substance into an active metabolite. It has proven highly effective in assessing human risk (1).

Description of Test (Rationale for the selection of strains)

The test was developed by Bruce Ames, Ph.D. from the University of California-Berkeley. The test involves the use of several different genetically altered strains of Salmonella typhimurium, each with a specific mutation in the histidine operon (2). The test substance demonstrates mutagenic potential if it is able to revert the mutation in the bacterial histidine operon to the wild type and reestablish prototrophic growth within the test strain. This reversion also can occur spontaneously due to a random mutational event. If, after adding a test substance, the number of revertants is significantly greater than the spontaneous reversion rate, then the test substance physically altered the locus involved in the operon's mutation and is able to induce point mutations (2).

In order to increase the sensitivity of the test system, other mutations in the Salmonella are used (2). To insure a higher probability of uptake of test substance, the genome for the lipopolysaccharide layer (LP) is mutated and, therefore, larger molecules are allowed to enter the bacteria. Each strain has another induced mutation which causes loss of excision repair mechanisms. A mammalian microsomal enzyme system is incorporated since many chemicals are not by themselves mutagenic but have to be activated by an enzymatic process. These microsomal enzymes are obtained from livers of rats induced with Aroclor 1254; the enzymes allow for the expression of the metabolites which would occur in the mammalian system. This activated rat liver microsomal enzyme homogenate is termed S-9.

Description of Strains (History of the strains used method t monitor the integrity of the organisms, and data pertaining current and historical control and spontaneous reversion rat

The test consists of using five different strains of ella typhimurium that are unable to grow in absence of histidine e of a specific mutation in the histidine operon. This histidine requirement is verified by attempting to grow the tester strains on minimal glucose agar (MGA) plates, both with and without histidine. The dependence on this amino acid is shown when growth occurs only in its presence. The plasmids in strains TA 98 and TA 100 contain an ampicillin resistant R factor. Strains deficient in this plasmid demonstrate a zone of inhibition around an ampicillin impregnated disc. The alteration of the LP layer allows uptake by the Salmonella of larger molecules. If a crystal violet impregnated disc is placed onto a plate containing any one of the bacterial strains, a zone of growth inhibition will occur because the LP layer is altered. The absence of excision repair mechanisms can be determined by using ultraviolet (UV) light. These mechanisms function primarily by repairing photodimers between pyrimidine bases. Exposure of bacteria to UV light will activate the formation of these dimers and cause cell lethality, since excision of these photodimers can not be made. The genetic mutation resulting in UV sensitivity also induces a dependence by the Salmonella to biotin. Therefore, this vitamin must be added. In order to prove that the bacteria are responsive to the mutation process, positive controls are run with known mutagens. If after exposure to the positive control substance, a revertant count is obtained which is greater than twice the spontaneous reversion rate, then the bacteria are adequately responsive. Sterility controls are performed to determine the presence of contamination. Sterility of the test compound is also confirmed in each first dilution. Verification of the tester strains occurs simultaneously with the running of each assay. The value of the spontaneous reversion rate is obtained by using the same inoculum of bacteria that is used in the assay (3).

Strains were obtained directly from Dr. Ames, University of California-Berkeley, propagated and then maintained at -80°C in our laboratory. Before any substance was tested, quality controls were performed on the bacterial strains to establish tne presence of their special features and also to determine the spontaneous reversion rate (2). Records are maintained of all the data to determine if deviations from the set trends have occurred. These records are kept in the archives of the Quality Assurance Unit.

In this series of tests for the detection of mutagenic potential of different agents, we compared the spontaneous reversion values with our own historical values and those cited by Ames et al (2). Our conclusions are based on the spontaneous reversion rate compared to the experimentally induced rate of mutation. When operating effectively, these strains detect substances that cause base pair mutations (TA 1535, TA 100) and frameshift mutations (TA 1537, TA 1538, and TA 98).

Objective of Study

The objective of the study is to determine the mutagenic potential of 4-nitrophenyl monochloromethyl (phenyl) phosphinate (CMP) by using the Ames Assay. Tester strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 were used. The test substance was dissolved in dimethyl sulfoxide (DMSO) and this diluent was checked for sterility.

METHODS (3)

Rationale for Dosage Levels and Dose Response Tabulations

To insure readable and reliable results, a sublethal concentration of the test substance had to be determined. This toxicity level was found by using MGA plates, various concentrations of the substance, and approximately 10^8 cells of TA 100 per plate, unless otherwise specified. Top agar containing trace amounts of histidine and biotin were placed on MGA plates. TA 100 was used because it is the most sensitive strain. Strain verification was confirmed on the bacteria, along with a determination of the spontaneous reversion rate. After incubation, the growth was observed on the plates. (The auxotrophic Salmonella will replicate a few times and potentially express a mutation. When the histidine and bi tin supplies are exhausted, only those bacteria that reverted to the prototrophic phenotype will continue to reproduce and form macrocolonies; the remainder of the bacteria comprises the background lawn. The minimum toxic level is defined as the lowest serial dilution at which decreased macrocolony formation, below that of the spontaneous revertant rate, and an observable reduction in the density of the background lawn occurs.) A maximum dose of 1 mg/plate is used when no toxicity is observed. The densities were recorded as normal, slight, and no growth.

Test Format

After we validated our bacterial strains and determined the optimal dosage of the test substance, we began the Ames Assay. In the actual experiment, 0.1 ml of the particular strain of Salmonella (10^8 cells) and the specific dilutions of the test substance were added to 2 ml of molten top agar, which contained trace amounts of histidine and biotin. Since survival is better from cultures which have just passed the log phase, the Salmonella strains are used 16 hours (maximum) after initial inoculation into nutrient broth. The dose of the test substance spanned a 1000-fold, decreasing from the minimum toxic level by a dilution factor of 5. All the substances were tested with and without S-9 microsome fraction. The optimal titer of the S-9 was determined and 0.5 ml was added to the molten top agar. After all the ingredients were added, the top agar was mixed, then overlaid on minimum glucose agar plates. These plates contained 2% glucose and Vogel Bonner "E" Concentrate (4). The water used in this medium and all reagents came from a polymetric system. Plates were incubated, upside down in the dark at 37° C for 48 hours. Plates were prepared in triplicate and the average revertant counts were recorded. The corresponding number of revertants obtained was compared to the number of spontaneous revertants; the conclusions were recorded statistically. A correlated dose response is considered necessary to declare a substance as a mutagen. Commoner (5), in his report, "Reliablilty of Bacterial Mutagenesis Techniques to Distinguish Carcinogenic and Non-Carcinogenic Chemical," and McCann et al (1) in their paper, "Detection of Carcinogens as Mutagen in the Salmonella/Mammalian Microsome Mutagenicity Test: Assay of over 300 Chemicals," have concurred on the test's ability to detect mutagenic potential.

Statistical Analysis

Quantitative evaluation was ascertained by the method of Ames et al (2). They assumed that a compound which causes twice the spontaneous reversion rate and a correlated dose response is mutagenic.

Chemical Analysis

Information on the chemical analysis of CMP appears in Appendix A.

RESULTS AND DISCUSSION

Throughout this report, the test substance will be referred to by its code number.

Substance	Code No.
4-Nitrophenyl Monochloromethyl (Phenyl) Phosphinate	CMP

On 10 August 1982 the toxicity level determination was performed for CMP. Results appear in Tables 1 to 5, Appendix B. For this experiment, all sterility, strain verification and negative controls were normal (Table 1). No toxicity was observed at the 1 mg/plate concentration (Table 2). Therefore, we use 1 mg/plate as the highest dose.

On 18 August 1982, the Ames Assay was performed on the test substance. In this assay normal results were observed for all sterility and strain verification controls (Table 3). Normal results were also observed for all positive and negative controls (Table 4). Following exposure of the bacteria to the test substance, no incidences of mutagenicity were observed (Table 5).

CONCLUSIONS

The Ames Assay is able to detect frameshift and basepair mutagenic potential. Our results show no evidence of such potential. Therefore on the basis of the Ames Assay, CMP both in the presence and absence of metabolic activation is not mutagenic at the levels tested.

RECOMMENDATION

CMP should be tested using other toxicological assays, if efficacy tests prove this compound to be a promising antidote.

REFERENCES

1. McCann JE, Choi E, Yamasaki E, Ames BN. Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proc Nat Acad Sci, USA 1975;72:5135-5139.

2. Ames BN, McCann J, Yamasaki E. Methods for detection carcinogens and mutagens with Salmonella/mammalian microsome mutagenicity test. Mutation Res 1975;31:347-364.

3. LAIR SOP OP-STX-1, Ames Salmonella/mammalian microsome mutagenicity test, 15 February 1982.

4. Vogel HJ, Bonner DM. Acetylornithinase of E. coli: Partial purification and some properties. J Biol Chem 1956;218:97-106.

5. Commoner B. Reliability of the bacterial mutagenesis techniques to distinguish carcinogenic and non-carcinogenic chemicals. EPA 600/1 76-022, 1976.

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APPENDICES

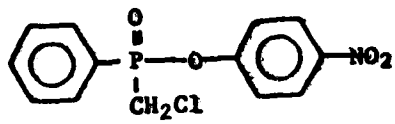
CHEMICAL ANALYSIS

Chemical Name: 4-nitrophenyl monochloromethyl (phenyl) phosphinate (CMP)

CAS: none

Molecular Formula: $C_{13}H_{11}ClNO_4P$

Molecular Structure:



Elemental Analysis:

	Calculated	Found
C	50.09	49.95
H	3.56	3.57
N	4.50	4.47
Cl	11.38	11.26
P	9.94	10.00

Molecular Weight: 311.67

Stability: base sensitive

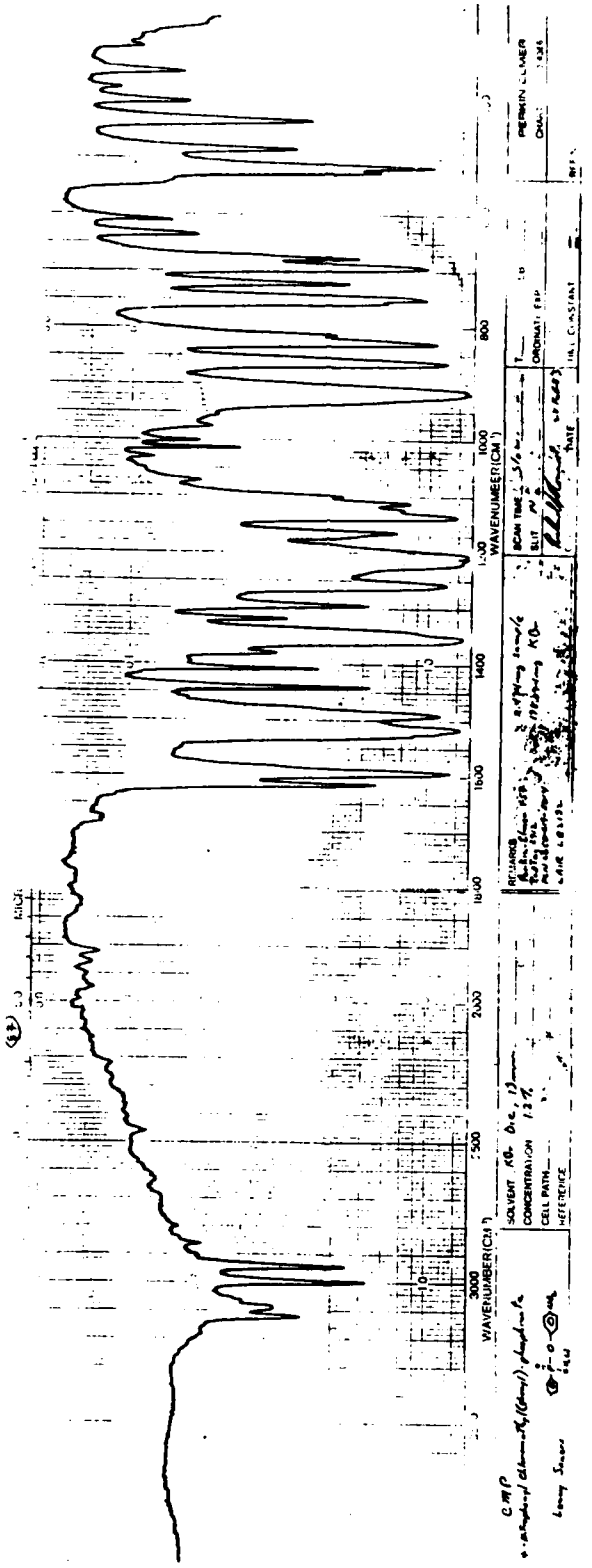
Appearance: white crystalline solid

Mp. obs: 77-78.5 C

IR spectra: attached

Manufacturer: Ash Stevens, Detroit Research Park,
5861 John C. Lodge Freeway,
Detroit, Michigan 48202

Manufacture Lot No.: MP-07-201



APPENDIX A (cont.)

LIST OF TABLES

APPENDIX B

TABLE 1

STRAIN VERIFICATION FOR TOXICITY LEVEL DETERMINATION

Strains	Histidine Requirement	Ampicillin Resistance	Sensitivity to UV	Sensitivity to Crystal Violet	Sterility Control	Response (1)
100	NG	G	NG	14.5 mm	NG	+
1537	NG	14.5 mm	NG	13.2 mm	NG	+
WT	G	NA	G	G	NA	+

STERILITY CONTROL

His-Bio Mix Initial: NG End: NG MGA Plate: NG

Top Agar Initial: NG End: NG

Diluent: NG Nutrient Broth: NG

Test Compound (a) CMP-NG (b) CHR6-NG (c) NA (d) NA (e) NA

G = Growth NG = No Growth NT = Not Tested NA = Not Applicable WT = Wild Type

Spontaneous Revertants: TA 100, No S-9 109, 95, 81, 97, 121, 109 Average: 102

(1) + = expected response - = unexpected response

Study Number: 82024 Date: 10 Aug 82 By: Sauers

APPENDIX B

TABLE 2

TOXICITY LEVEL DETERMINATION

Substance assayed: CMP Substance dissolved in: DMSO

Study Number: 82024 Date: 10 Aug 82 Performed by: Sauers, Kellner, Mullen

TA 100 REVERTANT PLATE COUNT

Test Compound Concentration	Plate #1	Plate #2	Plate #3	Average	Background Lawn (1)
1 mg/plate	64	85	82	77	NL
0.1 mg/plate	95	82	75	84	NL
0.01 mg/plate	107	85	87	93	NL
0.001 mg/plate	83	84	95	87	NL
0.001 mg/plate	81	86	87	85	NL
0.0001 mg/plate	94	83	95	91	NL
0.00001 mg/plate	91	91	119	100	NL
0.000001 mg/plate	84	81	127	97	NL

(1) NG = No Growth ST = Slight Growth NL = Normal Lawn

TABLE 3

STRAIN VERIFICATION CONTROL

Strains	Histidine Requirement	Ampicillin Resistance	UV	Sensitivity to Crystal Violet	Sterility Control	Response (1)
98	NG	G	NG	12 mm	NG	+
100	NG	G	NG	12 mm	NG	+
1535	NG	NA	NG	14 mm	NG	+
1537	NG	14 mm	NG	13 mm	NG	+
1538	NG	NA	NG	12 mm	NG	+
WT	G	NA	G	G	NA	+

STERILITY CONTROL

His-Bio Mix Initial: NG End: NG Diluent: NG

Top Agar Initial: NG End: NG MGA Plate: NG

S-9 Mix Initial: NG End: NG Nutrient Broth: NG

Test Compound (a) CHR4-NG (b) CHR6-NG (c) CMP-NG (d) NA (e) NA (f) NA

G = Growth NG = No Growth NT = Not Tested NA = Not Applicable WT = Wild Type

Study Number: 82024 By: Sauers

Date: 20 Aug 82

(1) + = expected response
- = unexpected response

TABLE 4

NUMBER OF REVERTANTS/PLATE

Compd.	Amount of Compd. Added	S-9 Added	Strain No. 98	100	1535	1537	1538
AF	2 ug/plate	yes	(648,518,591) 586	(337,377,285) 333			(579,677,449) 568
BP	2 ug/plate	yes	(94, 73, 90) 86	(350,361,357) 356		(37, 54, 27) 39	(76, 52, 87) 72
AA	2 ug/plate	yes	(612,803,656) 690	(999,831,934) * 921		(150,161,171) 161	(909,919,721) 850
MNNG	2 ug/plate	no		(871,999,999) * 956			
	20 ug/plate	no			(999,999,999) * 999		
Spontaneous Reversion Rate							
	before	yes	(18, 18, 25)	(89, 91, 74)	(9, 8, 8)	(3, 4, 5)	(15, 19, 11)
	after		(16, 19, 18) 19	(93, 92, 89) 88	(17, 13, 9) 11	(6, 5, 3) 4	(9, 12, 22) 15
	before	no	(11, 11, 23)	(77, 60, 69)	(11, 12, 7)	(3, 4, 7)	(14, 7, 10)
	after		(16, 17, 25) 17	(63, 87, 75) 72	(15, 10, 16) 12	(4, 3, 6) 5	(13, 15, 10) 12

* 999 = signifies a value greater than 1000

Study Number: 82024

Date: 20 Aug 82 By: Sauers, Kellner, Lewis, Dacey

TABLE 5

NUMBER OF REVERTANTS/PLATE

Compd.	Amount of Compd. Added	S-9 Added	Strain Number 98	100	1535	1537	1538
CMP	1 mg/plate	no	(10, 7, 10) 9	(50, 64, 62) 59	(9, 7, 9) 8	(3, 4, 3) 3	(8, 8, 12) 9
		yes	(17, 10, 7) 11	(54, 70, 72) 65	(5, 5, 5) 5	(2, 4, 3) 3	(19, 11, 16) 15
CMP	0.2 mg/plate	no	(10, 10, 14) 11	(50, 69, 65) 61	(11, 8, 7) 9	(3, 5, 6) 5	(8, 9, 11) 9
		yes	(14, 14, 21) 16	(64, 77, 68) 70	(10, 8, 7) 8	(5, 6, 2) 4	(12, 8, 17) 12
CMP	0.04 mg/plate	no	(7, 13, 12) 11	(73, 53, 61) 62	(7, 17, 10) 11	(2, 6, 4) 4	(5, 19, 11) 12
		yes	(25, 19, 18) 21	(71, 64, 62) 66	(8, 5, 7) 7	(5, 4, 2) 4	(13, 13, 19) 15
CMP	0.008 mg/plate	no	(12, 10, 10) 11	(55, 54, 50) 53	(11, 7, 8) 9	(4, 3, 2) 3	(5, 11, 14) 10
		yes	(11, 14, 15) 13	(70, 65, 54) 63	(13, 13, 12) 13	(6, 8, 5) 6	(22, 14, 15) 17

-continued

Study Number: 82024 Date: 20 Aug 82 By: Sauers, Kellner, Lewis, Dacey

TABLE 5 (concluded)

NUMBER OF REVERTANTS/PLATE

Compd.	Amount of Compd. Added	S-9 Added	Strain Number 98	100	1535	1537	1538
CMP	0.0016 mg/plate	no	(13, 7, 11) 10	(63, 67, con) 65	(17, 7, 12) 12	(2, 2, 6) 3	(12, 7, 6) 8
		yes	(12, 20, 14) 15	(61, 72, 75) 69	(9, 9, 8) 9	(4, 4, 6) 5	(14, 15, 14) 14
CMP	0.00032 mg/plate	no	(25, 21, 15) 20	(98, 93, 131) 107	(10, 13, 10) 11	(3, 3, 5) 4	(7, 8, 11) 9
		yes	(14, 11, 12) 12	(65, 79, 57) 67	(16, 9, 13) 13	(4, 2, 5) 4	(18, 12, 10) 13

con = plate value disregarded due to contamination

Study Number: 82024 Date: 20 Aug 82 By: Sauers, Kellner, Lewis, Dacey

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